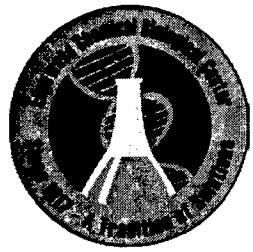

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ECBC-TN-019

CHARACTERISTICS AND SAMPLING EFFICIENCIES OF TWO IMPACTOR BIOAEROSOL SAMPLERS: MAS-100® (MICROBIAL AIR MONITORING SYSTEM) AND SINGLE-STAGE ANDERSEN VIABLE MICROBIAL SAMPLERS

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RESEARCH AND TECHNOLOGY DIRECTORATE

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Sandra J. Johnson
SANDRA J. JOHNSON
Chief, Technical Releases Office

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4. TEST PROCEDURES AND ANALYSIS

4.1 Sampling Efficiency Measurements.

The samplers and corresponding reference filters sampled the air simultaneously for the same amount of time. Tests with no aerosols were conducted to determine the background fluorescence of the samplers as well as reference filters.

Sampling efficiency tests were conducted with two kinds of aerosols and processing methods. The first method used monodisperse fluorescent PSL microspheres. The second method used monodisperse fluorescent oleic acid particles. Aerosol generation and processing methods are described in detail below.

4.2 Polystyrene Latex Microsphere (PSL) Tests.

Sampling efficiency tests were conducted with 1 and 2 μm blue fluorescing PSL microspheres and 3 μm green fluorescent PSL microspheres (Duke Scientific, Corporation, Palo Alto, CA). The PSL aerosol was generated using a 24-jet Collison nebulizer then passed through a radioactive isotope (Kr-85) neutralizer to reduce the charge on the particles. During the experiment, the aerosol was generated for 10 min, and mixed for 1 min before sampling.

The samplers and the corresponding reference filters sampled the PSL aerosol simultaneously for the same amount of time. Polycarbonate membrane filters (Osmonics Incorporated, Minnetonka, Minnesota) were used as reference filters to collect the fluorescent PSL microspheres. Impactors had wetted membrane filters on the impaction surface to collect particles. After sampling, the sample filters and reference filters were collected. The membrane filters were processed to remove microspheres from the filters into the liquid for fluorometer analysis (Kesavanathan and Doherty 1999). The removal procedure consisted of placing the membrane filters into filtered deionized water, hand shaking for 10 sec followed by vortexing for 50 sec. The hand shaking and vortexing for 60 sec were repeated four times (total of 5 min) to completely remove fluorescent PSL microspheres from the membrane filters.

4.3 Sodium Fluorescein Tagged Oleic Acid (Fluorescent Oleic Acid) Tests.

Sampling efficiency tests were also conducted with 4, 5, 7, and 8-μm fluorescent oleic acid particles. The monodisperse fluorescent oleic acid particles were generated using a Vibrating Orifice Aerosol Generator (VOAG, TSI Incorporated, St. Paul, MN). As with the PSL tests, the generated aerosol was passed through a Kr–85 radioactive isotope neutralizer to reduce charge on particles and then delivered to the chamber. Sampling the aerosol onto a microscope slide inserted into an impactor and measuring the droplet size using a microscope determined sizes of the fluorescent oleic acid particles. The measured fluorescent oleic acid particle diameter was converted to an aerodynamic particle size using a spread factor (Olan-Figueroa et al. 1982) and the density of fluorescent oleic acid. At the end of aerosol generation, the aerosol in the chamber was mixed for 1 min before sampling. The samplers and the corresponding

reference filters sampled the aerosol simultaneously for the same amount of time. Glass fiber filters (Pall Corporation, Ann Arbor, MI) were used as the reference filters to collect fluorescent oleic acid particles.

The glass fiber filters were removed from filter holders and samplers, placed into a fluorescein recovery solution, and gently shaken on a table rotator (Lab-Line Instruments, Incorporated, Melrose Park, IL) for 1 hr. The recovery solution used in the tests had water with a pH between 8 and 10, which was obtained by adding a small amount of NH_4OH (e.g., 1000 mL of water with 0.563 mL of 14.8 N NH_4OH). Factors that affect fluorescein analysis and the removal of fluorescein from filters are described in detail in an earlier publication (Kesavan et al., 2001). The fluorescence of the solution was measured using a fluorometer. All the samples were analyzed the same day as the experiment or the next day.

4.4 Analysis.

The sampling efficiency was determined by comparing the fluorometer-measured fluorescence of the sampler filter to the reference filters. The airflow rate of the samplers and reference filters and the liquid volume of their processing solutions were taken into account in the calculation.

5. RESULTS

The sampler characteristics for the two samplers are shown in Table 1 and efficiency results are shown in Table 2. A graph of the efficiency results of the MAS-100® is shown in Figure 5, and the efficiency of the Andersen sampler is shown in Figure 6.

Table 2. Sampling Efficiency (%) of Samplers by Particle Size

Particle Size (μm)	Sampling Efficiency (%) ± one standard deviation	
	MAS-100®	Single-Stage Anderson
1	7.3 ± 2.2	57.5 ± 11.2
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4	68.4 ± 2.5	88.5 ± 12.3
5	66.1 ± 5.3	
7	61.7 ± 0.9	
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14. ABSTRACT
Sampler characteristics and efficiencies of two impactor type biological air samplers were determined. Both impactors are based on the principle of air accelerating through an entry plate with critically sized orifices and inertially impacting onto a surface below. A petri dish with agar is used as the impaction surface for these samplers. The MAS-100® is a single-stage impactor that aspirates air through a 400-hole perforated entry plate onto an agar plate at an airflow rate of 100 L/min. The single-stage Andersen impactor aspirates air through a 400-hole perforated entry plate onto an agar plate at an airflow rate of 28.3 L/min (1 cfm). The sampling efficiency tests were conducted using polystyrene latex microspheres and sodium fluorescein tagged oleic acid particles. The analysis was by fluorometry. The MAS-100® can be powered using its internal rechargeable battery or 36 Watt AC power. The Andersen impactor power requirement depends on the vacuum pump that draws air through the unit. The sampling efficiency results showed that the MAS-100® has a peak of 68.4% ± 2.5 for 4 μm particles, and the single-stage Andersen has a peak of 88.5% ± 12.3 for 4 μm particles, compared to reference filter samples.

15. SUBJECT TERMS

Aerosol	Samplers	Petri dish
Impactor	Biological	Sampling efficiency

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PREFACE

The work described in this report was authorized under Project No. 622384/ACB2. The work was started in March 2002 and completed in October 2002.

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1. INTRODUCTION

Air samplers are used to detect the presence of chemical, biological, and nuclear aerosols. Samplers and detection systems must be tested and their performance efficiencies defined. Knowledge of equipment performance enhances the ability to protect soldiers, first responders, and the general public.

Air samplers for biological material must collect them in a gentle manner to reduce destruction to the organism if the analysis method requires the organism to be alive. Vegetative bacteria may be killed if collected dry. To reduce the drying of the organism (dessication), samplers may collect biological material in liquid or nutritionally appropriate agar type media. An ideal biological sampler should be small, portable, use minimal power, have a high sampling efficiency, and preserve the viability of the organism.

In this study, characteristics and sampling efficiencies of two samplers were evaluated: MAS-100® Microbial Air Monitoring System [Merck Eurolab/Brussels and EM Science (EMD)/Gibbstown, NJ] and a single-stage Andersen Viable Microbial Sampler (Thermo Andersen, Smyrna, GA). Each sampler's characteristics, such as size, weight, airflow rate, and power consumption were measured. Sampling efficiency experiments were conducted in a 70-m^3 chamber at the U.S. Army Edgewood Chemical Biological Center (ECBC) using fluorescent polystyrene latex (PSL) microspheres and sodium fluorescein tagged oleic acid (fluorescent oleic acid) particles. Typically, four tests were performed for each particle size (on each piece of equipment). Where results appear anomalous, repeat tests were performed, as was the case with the MAS. The final tally of activity shows that 50 tests were performed to characterize the two samplers: 36 tests on the MAS for six particle sizes and 14 tests on the Andersen 1-Stage for four particle sizes.

The performance of an aerosol sampler is the product of the sampler's aspiration, transmission, and collection efficiencies. The aspiration efficiency of a sampler gives the efficiency with which particles enter into the sampler inlet. Transmission efficiency gives the efficiency with which the particles are transported to the collection point. The collection efficiency gives the efficiency with which particles are captured and retained by the sampling medium. In this study, the samplers were tested in an environmentally controlled chamber at calm air conditions and did not include inlet efficiencies at varying wind velocities.

In this study, the sampler characteristics and sampling efficiency measurements of two impactor air samplers with agar plates were determined. Both impactors are based on the principle of air accelerating through an entry plate with critically sized orifices and inertially impacting onto a surface below the perforated entry plate. The inertial impaction theory was described in 1952 by Ranz and Wong (ThermoAndersen 2003) and has been utilized in

Andersen impactors for approximately 30 years. For biological sampling, the MAS-100® and the Andersen impactor collect particles onto petri dishes filled with agar.

Although basic physical principle of particles may be broadly applied to sampling for biological aerosols, there are a number of complicating factors that affect the performance of an aerosol sampler with different specific bioaerosols.

Bioaerosols have a number of characteristics that may affect their sampling. Bioaerosol particles may be single spores pollen grains, bacterial cells or viruses, aggregates of spores or cells, fragments, or biological particles carried by non-biological material. Particle shape affects its aerodynamic behavior in a sampler, which determines collection efficiency. Bioaerosol particles have many different shapes. For example, bacteria may be shaped spherical, rod-shaped, spiral, or filamentous. Spherical bacteria may occur as pairs, tetrads, or clusters, thus affecting their overall size (and collection efficiencies of different samplers vary by size). For example, aggregation of a 7 μm bacterial particle may make it larger than the optimal collection size for that sampler. Thus, it is important to be specific about not just the size of the biological particles of interest, but also of its behavioral/physical quirks. When airborne, bacteria occur as aggregates or microcolonies attached to materials, it again changes the particle size and may make it difficult to predict which sampler would be best to use (Willeke and Baron 1993).

Bioaerosol particles vary over a range of sizes. Viruses may be 0.02-0.3 μm in length, whereas bacteria and fungal spores range from about 0.3 to 100 μm. Pollen, algae, and protozoa may be tens to hundreds of micrometers in diameter. Their collection will vary depending on whether they are collected as individual cells or spores or are aggregated onto other materials (Willeke and Baron 1993). Concentration level of the aerosol should also be considered. Culture-plate impactors may readily overload unless the medium is processed for dilution culture. Other aerosols not relevant to the study may also deposit on the impaction surface (Burge 1995).

Viability (able to reproduce and/or have metabolic activity) of a sampled organism may be affected by ultraviolet light, temperature, dryness, toxic gases, and sampling stresses such as shear forces. Some organisms such as fungal spores and pollen tend to be hardier due to nature's design to allow transmission to other areas. Vegetative cells of bacteria tend to be damaged more easily by environmental and sampling stresses (Willeke and Baron 1993).

Culture media or adhesive surface onto which the organism is collected are also critical factors in successful sampling of biological organisms. Nutritional requirements of some organisms are not well understood and may affect the interpretation of sampling results (Willeke and Baron 1993). Sampling times must be adjusted to avoid dehydration of the sampling media or dessication of the biological organism. Media composition may need to be adjusted to allow some fluid loss during sampling. If the sampling media is too liquid/watery, penetration of the media by the biological organism may alter the Colony Forming Units (CFU) result. Embedding the biological organism into the media may change its access to air and light (Burge 1995).

In conclusion, expected physical and biological variations of the organism must be defined, and the nutritional and environmental needs must be considered to select optimal media and handling/storage/incubation.

2. CHAMBER

Sampler characterization tests were conducted in a 70 m^3 bio-safety Level 1 chamber that has ultra-violet light sources to kill biological material. Temperature and humidity of the chamber can be set and maintained easily and accurately by a computer. This computer also controls power receptacles inside the chamber.

HEPA filters are installed at the inlet to filter air entering the chamber to achieve very low particle concentrations in the chamber. Similarly, HEPA filters are installed at the exhaust port to filter all particles leaving the chamber. Exhausting chamber air through the HEPA filters and pumping HEPA filtered air into the chamber reduces the aerosol concentration in the chamber. The maximum amount of airflow that can be exhausted from the chamber is approximately 700 cubic ft/min (approximately $2x10^4$ L/min) by the exhaust pump. There is also a small re-circulation system that removes air from the chamber, passes it through a HEPA filter, and delivers it back to the chamber. This system is useful when the aerosol concentration in the chamber needs to be reduced by a small amount.

Aerosols can be generated outside and delivered to the chamber or can be generated inside the chamber. A fan mixes the chamber air after and/or during the aerosol generation to achieve uniform aerosol concentration in the chamber. Previous tests showed that mixing the aerosol in the chamber for 1 min is adequate to achieve uniform aerosol concentration.

3. SAMPLERS

Two impactor style samplers were tested to determine sampling efficiency. The MAS-100® was tested using an airflow rate of 100 L/min. The single-stage Andersen impactor was tested using an airflow rate of 28.3 L/min (1 cfm). The sampling efficiency tests were conducted using 1, 2, and 3 μm polystyrene latex (PSL) microspheres and 4, 5, 7, and 8 μm sodium fluorescein tagged oleic acid (fluorescent oleic acid) particles. The analysis was by fluorometry. The MAS-100® can be powered using its internal rechargeable battery or AC power at a maximum 36 W. The Andersen impactor power requirements depend on the vacuum pump, which draws air through the unit. These tests used ¼ hp Gast Manufacturing vacuum pumps, 4.8-5.0 A with the Anderson impactor.

3.1 Collection Media.

Glass petri dishes were used to avoid the possible influence of static charge developed by airflow over plastic dishes. Agar was found to fluoresce at the wavelengths evaluated, so melted paraffin wax was used as the collection substrate. The wax was melted, then poured into the glass petri dish (Figure 1) to a total height of approximately 8 mm from the

surface of each support plate (including the bottom thickness of the dish). A white, 0.8 μm pore size polycarbonate membrane filter, 85-mm diameter, was placed onto the solid wax surface (Osmonics Incorporated, Minnetonka, MN). Two-milliliters of filtered deionized water was dispersed onto the filter to hold it in place on the wax surface and minimize particle bounce during testing. For optimal consistent collection results, it is important to have a level surface on the collection media. Careful control of hot liquid media temperature and cooling time can prevent a concave surface from forming in the center of the media; an additional factor is that the media needs to flow evenly to meet the sidewalls of the dish (not curling down leaving a gap between the media and the glass wall).

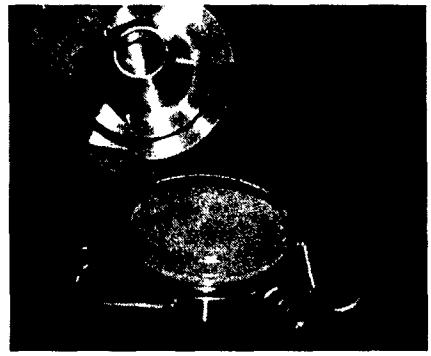

Figure 1. Glass Petri Dish Filled to Depth of 8 mm with Paraffin Wax in Single-Stage Andersen Impactor

3.2 MAS-100®.

A picture of the MAS-100® is shown in Figures 2 and 3. The unit is cylindrical, relatively small in size, and quiet during operation. Physical dimensions of the MAS-100® (Merck Eurolab) are: 10.25 in. height, 4.3 in. diameter, and weighs 4.85 lb (including built-in rechargeable battery).

Figure 2. MAS 100® Single-Stage Impactor. Top view with wax-filled glass petri dish (open).

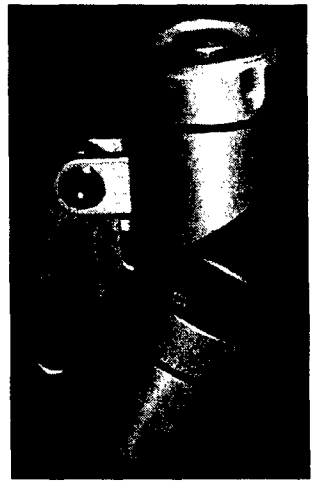

Figure 3. MAS-100® Single-Stage Impactor. Side View

The MAS-100® is based on the principle of the single-stage Andersen impactor, using inertial impaction as the collection mechanism. It operates by aspirating air through a 400-hole perforated entry plate onto a petri dish containing biological collection media (e.g., agar), at 100 L/min airflow rate. According to manufacturers literature, this enables an impaction velocity of approximately 11 m/s (approximately 36 ft/sec). This velocity was selected to optimize collection of particles >1 μm diameter. The manufacturer states that any standard size petri dish could be used and differences in the petri dish fill should not affect airflow. Petri dishes should be at room temperature to avoid condensation. The MAS-100® can be used in manual or programmable mode and can interface with a personal computer. A small LCD display (32 character) is used for programmable functions and indicated battery charge level. The MAS-100® can be operated using either its self-contained rechargeable battery or AC power. Design specifications indicate that the MAS-100® has the capacity to aspirate approximately 50,000 L of air with a fully charged battery (at 100 L/min, that is approximately 8 hr 20 min), although 10 min maximum per petri dish is recommended to avoid dehydration of the agar.

3.3 Single-Stage Andersen Impactors.

Andersen impactors perform collection of airborne particles using inertial impaction as the collection mechanism. The inertial impaction theory was discussed in 1952 by Ranz and Wong and has been extensively used in Andersen impactors for approximately 30 years (ThermoAndersen 2003). These tests evaluated collection efficiency onto a petri dish (ThermoAnderson 2003). The Andersen impactor may be used to collect either airborne bacteria, fungus, or viral aerosols. Particles are impacted onto a collection surface, which may be either culture media in a petri dish or a filter placed on the collection stage. It is best if the collection surface (i.e., agar) is sticky to minimize particle bounce. The Andersen impactors are designed to use moderate airflow rates (28.3 L/min; 1 cfm), and the petri dishes may utilize any culture media (i.e., agar) as the collection substrate to culture different organisms. After sampling, the petri dish is removed, incubated, and CFUs are counted to determine the type and prevalence of the organisms. For this test series, new ring gaskets were placed on the single-stage Andersen impactor.

The inertial impaction collection mechanism is accomplished by drawing air through a series of holes (jets) in the collector. Each stage has 400 precision-machined jets. Larger particles, with more energy, deposit on the impaction surface and the smaller particles flow around the impaction surface. Aerodynamic diameter is important as it defines the behavior of large aerosol particles, including inertial properties, aerodynamic drag, and settling behavior. Size, shape, and density determine a particle's aerodynamic diameter. Because the jet size determines performance of the collection device, it is extremely important that the jets are thoroughly cleaned, with no material or residues in the holes.

The single-stage Andersen is used where total viable CFUs need to be determined, and size distribution is not required. The impactor is comprised of an inlet cone with an approximate 1-in. circular opening, and a single-stage with 400 jets for air to pass through then impact onto the collection surface (petri dish with media and filter for these tests). The Single-stage Andersen corresponds to Stage No. 6 of the six-stage Andersen Impactor.

The required airflow is 28.3 L/min (1 cfm). Airflow rate is critical to optimal performance of the Andersen impactors. This sampler is cylindrical in shape, 2.9 in. high, 4.13 in. diameter, and weighs 1.25 lb (without the pump). A separate air pump is necessary for using the Andersen sampler. This test used a ¼-hp vacuum pump (Gast Manufacturing, Incorporated, Benton Harbor, MI) with a power requirement of 4.8-5.0 A. A picture of the single-stage Andersen impactor is shown in Figure 4.

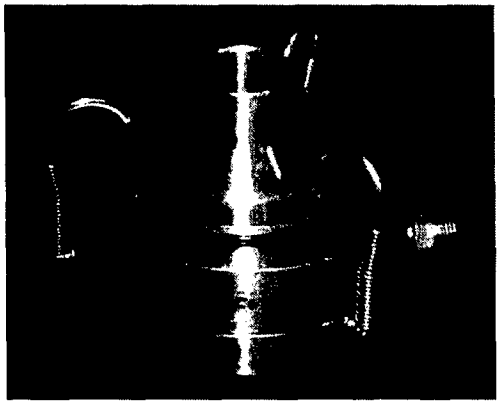

Figure 4. Single-Stage Andersen Impactor

3.4 Sampler Characteristics Measurements.

The airflow rates of the reference filters and Andersen samplers were measured using a Buck Calibrator (A.P. Buck, Incorporated, Orlando, FL). The MAS-100® was measured using a Kurz airflow meter (Kurz Instruments, Incorporated, Monterey, CA). The weight and dimensions of the samplers were measured, and the power usage was measured using a power meter (Extech Instruments, Taiwan). These measurements are listed in Table 1.

Table 1. Sampler Characteristics

Characteristics	MAS-100®	Single-Stage Andersen
Designed airflow rate (L/min)	100	28.3
Measured airflow rate at inlet (L/min)	100.2	28.3
Power (W)	36	depends on pump used
Physical parameters: Weight (lb) Height (in.) Diameter (in.)	 4.85 (w/battery) 10.25 4.3 (cylindrical shape)	 1.25 lb. (w/o pump) 2.9 4.13 (cylindrical shape)

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reference filters sampled the aerosol simultaneously for the same amount of time. Glass fiber filters (Pall Corporation, Ann Arbor, MI) were used as the reference filters to collect fluorescent oleic acid particles.

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The sampling efficiency was determined by comparing the fluorometer-measured fluorescence of the sampler filter to the reference filters. The airflow rate of the samplers and reference filters and the liquid volume of their processing solutions were taken into account in the calculation.

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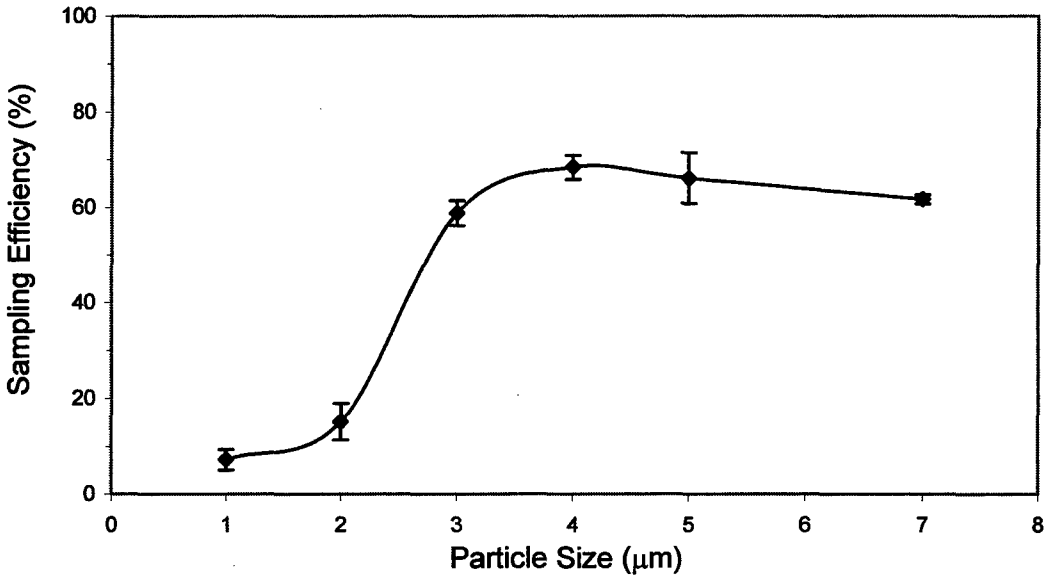

Figure 5. Sampling Efficiencies for the MAS-100® Impactor

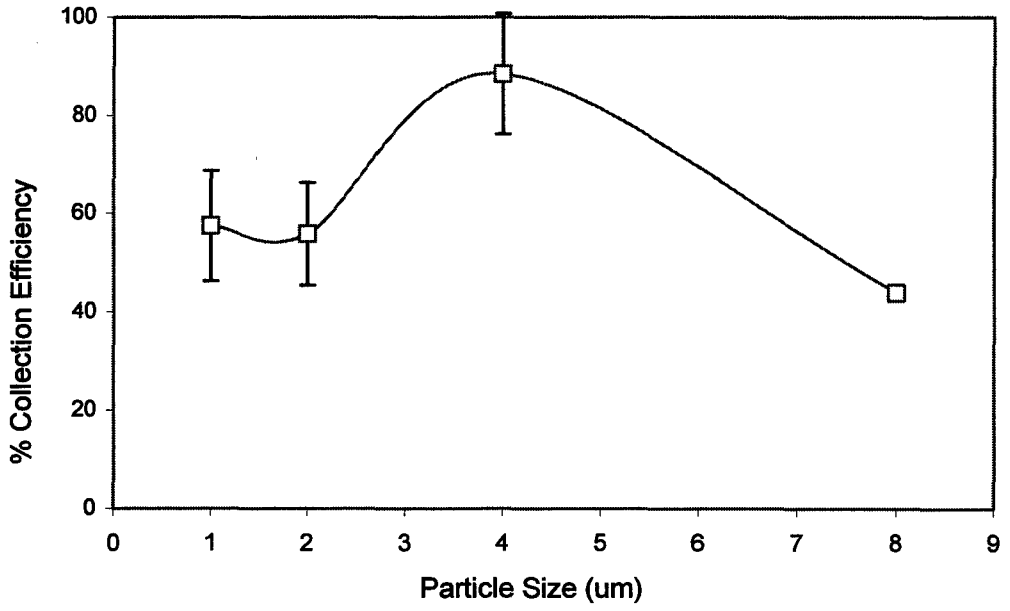

Figure 6. Sampling Efficiencies for the Single-Stage Andersen Impactor

The sampling efficiency results showed that the MAS-100® had a peak of 68.4% ± 2.5 for 4 μm and 66.1% ± 5.3 for 5 μm particles compared to the reference filter samples. The single-stage Andersen had a peak of 88.5% ± 12.3 for 4 μm particles.

For the single-stage Andersen impactor, theoretically 95% or more of the viable particles above 0.8 μ can be collected onto an agar surface. Efficiency test results were lower than the theoretical estimates (43.9 - 88.5%). The single-stage Andersen impactor showed large variability of test efficiency results for 1, 2, and 4 μm particles, shown by the error bars on Figure 5.

6. CONCLUSIONS

The MAS – 100® and single-stage Anderson impactors are bio-aerosol samplers that collect particles onto agar plates. Sampling efficiency tests were conducted using monodisperse 1, 2, and 3 μm PSL microspheres and 4, 5, 7, and 8 μm fluorescent oleic acid particles. The analysis was by fluorometry. For 1, 2, and 4 μm particles, the Andersen impactor performed better than the MAS-100®. Both impactors performed best with an approximate particle size of 4 μm. Sampling efficiency for 4 μm particles is 68.4% ± 2.5 for the MAS-100® and 88.5% ± 12.3 for the single-stage Anderson sampler.

7. RECOMMENDATIONS FOR FUTURE WORK

The MAS-100® has low sampling efficiency for small particles (1 μm). Therefore, experiments need to be conducted to determine the optimal flow rate to capture the small particles.

The sampling efficiency of larger (>4 μm) particles is low in the single-stage Andersen impactor. More tests need to be conducted with larger particle sizes to determine the reason for the low sampling efficiency.

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